739-Pos Board B539
Self Assembled Porphyrin-DNA Antenna Complex
Jakob G. Woller, Karl Börjesson, Bo Albinsson.
Fluorescent intercalating dyes have previously been used to form a self-assembled photonic wire at the nano scale, employing long range energy transfer. Here we present a mimic of a photosynthetic reaction centre antenna complex using porphyrin-modified DNA and fluorescent intercalating dyes. Visible light is used to excite an intercalated dye followed by fast energy transfer between dyes due to their large spectral overlap. A redox active porphyrin moiety absorbing at higher wavelength acts as an energy sink, thereby transferring energy from the DNA strand to the porphyrin.

Effectively this energy transfer process has increased the absorption coefficient of the porphyrin. We will study the efficiency of energy transfer to the porphyrin as a function of intercalating dye concentration, and study the effect of lipid vesicles on the system.

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Development of Fluorescently Tagged BMP-2 analog
Hemanth Akkiraju, Jeremy Bonor, Anja Nohe.
Our objective was to study cell signaling pathways initiated by Bone Morphogenetic Protein-2(BMP-2) by live cell imaging techniques. In order to achieve this we developed a novel method to covalently link Quantum Dots (QDs) to BMP-2 using N,N′-dicyclohexylcarbodiimide (DCC) as the cross linker .QD's are semi-conducting nanocrystals superior to the conventional organic dyes due to their high extinction coefficient and their stability (minimizing photobleaching), which allows it to be 20 times brighter and 100 times more stable than traditional fluorescent reporters. Quantum dots research in biological applications can be used to test for understanding ligand receptor interactions on the cell surface and its signaling pathways for growth factors like BMP-2. BMP-2 has been recognized by the FDA to be used for therapeutic treatments playing a key role in osteogenesis through cell differentiation. We know that BMP-2 binds to type I and type II receptors inducing phosphorylation to initiate Smad 1,5 and 8 signaling pathways for differentiation. To clearly track this phenomenon, using fluorescently tagged BMP-2 to quantum dots is the first step in prospect to study the signaling pathway in real time imaging to redefine the dynamics of ligand receptor binding during differentiation. Conjugating QD's with BMP-2 we ran stability tests using Fourier Transform Infrared Spectroscopy (FTIR) to identify the time scale for the protein degradation. These approaches should permit fluorescent BMP-2 in live cell imaging through microscopic techniques in vitro and in vivo to elucidate BMP-2 dynamics to initiate signaling pathways.

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Herzberg-Teller Vibronic Contribution to Mesomeric Dipole Moment Determines Two-Photon Absorptivity of Fluorescent Proteins
Mikhail Drobizhev, Nikolay S. Makarov, Shane Tillo, Thomas E. Hughes, Aleksander Rebane.
Fluorescent proteins (FPs) are widely used in two-photon microscopy as genetically-targeted bio-probes. The physical basis of large variability of their two-photon absorption (2PA) brightness is however not understood.
We have recently demonstrated that the mFruits series of FPs, having the same red anionic chromophore, show the 2PA band in the region of S_0 - S_1 electronic transition, corresponding to 900 - 1200 nm of laser wavelength. In this 2PA band, the vibronic 0-1 transition is stronger than the 0-0 transition, in contrast to one-photon absorption spectrum where the 0-0 transition dominates. It is also intriguing that the strength of the dominant vibronic 2PA transition strongly depends on the surrounding of the chromophore.
Here we perform a comprehensive analysis of the 2PA spectral profiles of Fruits FPs. We show that a crucial factor which drives their optical properties is the local electric field at the chromophore site (varying from one mutant to another). Variation of the field promotes the shift of equilibrium between the two resonating forms of the chromophore π-conjugation structure, which, in turn, results in systematic changes of mesomeric dipole moment (the difference between the dipole moments in the excited and ground states, $\Delta\mu$) and of the single-to-double bond-length alternations (BLA).
Because the two-photon tensor of the S_0 - S_1 transition is proportional to $\Delta\mu^2$, wesuggest an interesting physical effect implying strong Herzberg-Teller coupling of $\Delta\mu$ with the BLA coordinate. This effect can only be observed in 2PA spectrum.
Our model quantitatively explains the vibronic enhancement in 2PA spectrum of Fruits FPs and also provides the upper limit estimation for the 2PA peak cross section of any FP possessing red anionic chromophore. It also can guide mutagenesis efforts toward improvement of two-photon brightness of FPs.

742-Pos Board B542
Maximizing Photon Counts and Time Resolution for Single Molecule Fluorescence Studies in Micro Fluidic Mixing System
Denis Doerr, Ralf Kuenemuth, Shawn H. Pfeil, Everett A. Lipman, Claus A.M. Seidel.
Since the late 1990s microfabricated diffusive mixers have found applications in a growing number of chemical and biochemical kinetic assays. Microfabricated diffusive mixers require only microliters of sample, and use laminar flow to decouple laboratory time from reaction progress. These attributes make them ideally suited for kinetic measurements with single-molecule sensitivity. The time resolution is set by the time required to diffusively mix and the dwell time needed to insure a given number of photons. In addition, faster flow velocities are preferable to give minimum time uncertainty for the fixed position uncertainty of each measurement. One approach to obtain adequate signal at high flow is to increase the excitation intensity, however this alone is unsatisfactory since it increases the probability of populating long lived triplet and radical states. Here we show that the application of radical and triplet quenchers can be used to overcome this limitation. In fact, adequate data can be obtained from molecules spending as little as X ms in the detection volume. In addition, we show that using Fluorescence Correlation Spectroscopy (FCS) the velocity at each observed point can be measured, allowing the real time flow pattern to be obtained in parallel to molecular properties. This is more satisfying and less time consuming then fluid dynamics simulations, and allows for more accurate conversions between distance and time by accounting for variation in mixer fabrication and driving pressures.

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Microfluidic Mixing Device for Submilisecond Reactions
Leszek A. Gierusz.
Continuous-flow, together with pressure-jump and temperature-jump are effective methods of investigating very rapid reactions that occur in a submilisecond timescale, such as protein folding or fast enzyme kinetics.
Continuous-flow in particular has been successfully applied to the investigation of early events in protein folding pathways (Chan et al., 1997, Shastry et al., 1998, Matsumoto et al, 2007). Though effective, continuous-flow is not a widespread method, due to the lack of efficient and standardised mixers available commercially. Instead, the few existing instruments rely on hand-made devices designed, manufactured and assembled by dedicated research groups. This severely limits the availability of the continuous-flow method, to wider research applications.
Recent developments in microfabrication allowed to incorporate this highly effective method into continuous-flow mixer manufacturing. However lack of deeper understanding of fluid dynamics and related processes occurring within the mixer tend to be a limiting factor, confining continuous-flow mixer design to trial-and-error procedure rather than a carefully guided process. This project attempts to incorporate flow-dynamic simulations into mixer design in order to deliver an optimized continuous-flow mixing device suitable for mass production and commercialisation. We present progressive mixer designs backed up by flow-dynamic simulations and experimental results of an optimized mixer prototype.
Chan, C.K., Y. Hu, et al. (1997). ''Submillisecond protein folding kinetics studied by ultrarapid mixing.'' Proc. Natl. Acad. Sci. USA *94*: 1779–1784.
Matsumoto, S., A. Yane, et al. (2007). ''A rapid flow mixer with 11-μs mixing time microfabricated by a pulsed-laser ablation technique: observation of a barrier-limited collapse in cytochrome c folding.'' J. Am. Chem. Soc. *129(13)*: 3840–3841.
Shastry, M.C.R., S.D. Luck, et al. (1998). ''A continuous-flow capillary mixing method to monitor reactions on the microsecond time scale.'', Biophys. J. *74*: 2714–2721.

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Rapid Catch and Signal (RES) Platform Technology: Multiplexed Three-Color, Microwave-Accelerated Metal-Enhanced Fluorescence 20 Second DNA Assays
Anatoliy I. Dragan, Rada Pavlovic, **Chris D. Geddes**.
We present a new ''Rapid Catch and Signal'' (DNA-RCS) technology for the simultaneous highly selective detection of multiple DNA sequences in solution.